Smc5/6, an atypical SMC complex with two RING-type subunits

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ABSTRACT

The Smc5/6 complex plays essential roles in chromosome segregation and repair, by promoting disjunction of sister chromatids. The core of the complex is constituted by an heterodimer of Structural Maintenance of Chromosomes (SMC) proteins that use ATP hydrolysis to dynamically associate with and organize chromosomes. In addition, the Smc5/6 complex contains six non-SMC subunits. Remarkably, and differently to other SMC complexes, the Nse1 and Nse2 subunits contain RING-type domains typically found in E3 ligases, pointing to the capacity to regulate other proteins and complexes through ubiquitin-like modifiers. Nse2 codes for a C-terminal SP-RING domain with SUMO ligase activity, assisting Smc5/6 functions in chromosome segregation through sumoylation of several chromosome-associated proteins. Nse1 codes for a C-terminal NH-RING domain and, although it has been proposed to have ubiquitin ligase activity, no Smc5/6-dependent ubiquitylation target has been described to date. Here, we review the function of the two RING domains of the Smc5/6 complex in the broader context of SMC complexes as global chromosome organizers of the genome.

Introduction

Arranging a genome inside a cell is not an easy task. This is due to the exceptionally long, helical and fragile nature of the DNA molecule. In spite of this, cells need to physically manipulate their chromosomes at multiple levels to control different transactions, including expression and repression of genes, chromosome replication, sister chromatid segregation and DNA repair [1].

Various elements help to functionally organize and package DNA. One basic mechanism used by eukaryotic cells is to wrap DNA around a histone core to form nucleosomes and chromatin fibers. However, there is a family of protein complexes, more ancient than histones and present in all domains of life, which sustains chromosome organization at a larger scale than nucleosomes. They are known as Structural Maintenance of Chromosomes (SMC) complexes. Prokaryotic SMC complexes are mainly involved in chromosome partition by compacting and resolving sister chromatids during replication [2,3]. Eukaryotic cells have three main SMC complexes: cohesin, condensin and Smc5/6 [4]. Cohesin was initially discovered by its essential role in sister chromatid cohesion [5,6], but also participates in DNA repair and transcriptional regulation, by looping chromatin fibers and connecting distant regions of the genome [7,8]. Condensin participates in the structural organization of chromosomes, especially during mitosis, when chromatin fibers have to be disentangled and folded into compact chromosomes [9,10]. The essential function of the Smc5/6 complex is less well understood, although it seems to be involved in sister chromatid disjunction, particularly after blockage of replication forks [8,11].

General structure and molecular activities of SMC complexes

SMC proteins are rod-shaped molecules with two globular ends connected through an elongated coiled coil, known as ‘arm’ domain. One end of the protein codes for a ‘hinge’ domain, while the other end forms an ATPase ‘head’ domain (Figure 1A). Individual SMC proteins dimerize by interacting through the hinge domain and a kleisin subunit bridges the heads at the other end of the dimer [12]. The arm domain in the prokaryotic MukBEF and eukaryotic cohesin and condensin complexes is able to fold back at an elbow region, a coiled coil disruption present in all SMC complexes; this articulation brings the hinge domain halfway down the ATPase heads [13,14] (Figure 1B). Although not all SMC complexes may fold in exactly the same way, the presence of these disruptions indicates that the arm domain is not rigid. The interaction between the kleisin and the SMC heads is asymmetric [15–18]. The N-terminus of the kleisin interacts with the neck, a coiled-coil segment immediately adjacent to the ATPase head of one SMC; the C-terminus binds the ATPase head of the opposite SMC subunit [16–18]. The asymmetric nature of SMC complexes is more evident in eukaryotes, where two different SMC proteins heterodimerize at the core of an SMC complex, in contrast to SMC homodimers in prokaryotes.

Besides SMC and kleisins, all SMC complexes contain additional subunits. Most of them interact with the central part of the kleisin and the head domains and probably regulate the ATPase activity and the association of the complex with DNA. Structural analysis of kleisin-associated subunits allows the broad classification of SMC complexes in two main types (Figure 1B). The non-core subunits of condensin and cohesin are members of the ‘HAWK’ (HEAT proteins Associated With Kleisins) family [19]. On the other

hand, kleisin-associated subunits in prokaryotic and the eukaryotic Smc5/6 complexes belong to the 'KITE' family (Kleisin Interacting Tandem winged-helix Element) [20–22].

The ATPase activity of the heads is critical for the dynamic association of SMC complexes with DNA. Binding to ATP engages the heads, while its hydrolysis brings them apart (Figure 1C). An emerging picture is that these changes drive remodeling of distal parts in the molecule, including the arm domain, a feature that enables SMC complexes to dynamically associate with and organize DNA. Recent findings indicate that a common feature of all SMC complexes is their ability to organize chromosomes by extruding loops of DNA [23,24]. To this end, SMC complexes use (i) different domains to bind DNA and (ii) their ATPase activity to promote conformational changes, drive loop extrusion and entrap DNA in a topologically closed compartment. The heads, the hinges and other non-SMC subunits might serve as DNA-binding interfaces and anchor points. On the other hand, the conformational changes depend on the ATPase cycle: binding of ATP engages the ATPase heads and the SMC arms adopt an open conformation [25]; upon hydrolysis of ATP, the heads change their conformation, closing the arm space between the two SMC subunits [23,25] (Figure 1D). To allow the topological association of the complex with DNA, the ATPase activity needs to be exquisitely coordinated with opening of a protein-protein gate in the SMC complex, most probably at the kleisin-SMC interface [17,26,27]. Finally, the whole process must be very dynamic, allowing SMC complexes to alternate between different DNA binding interfaces and gradually expand loops.

Architecture and functions of the Smc5/6 complex

From the architectural point of view, there are several features that make Smc5/6 an atypical SMC complex. First, the arm domains are shorter than in cohesin or condensin. Second, Smc5/6 is equipped with a higher number of relatively small subunits, named non-SMC element (NSE) proteins. Third, the presence of KITE subunits (Nse1 and Nse3) reveals that the Smc5/6 complex is evolutionarily closer to prokaryotic SMC complexes than cohesin and condensin [20]. Fourth, the hinge domain in Smc5 and Smc6 include hub and latch regions important for binding to single stranded DNA [28]. Fifth, the Nse2 subunit directly binds to the middle of the arm domain in Smc5, an unusual characteristic for an SMC subunit. However, the most striking feature in Smc5/6 is the presence of two subunits with RING domains: Nse2 (also known as Mms21) contains an SP-RING (*S*iz-*P*IAS RING) SUMO E3 ligase domain and Nse1 has a NH-RING (*N*se1-*H*omologue RING) domain, highly related to ubiquitin E3 ligases [29,30] (Figure 2). Their presence points to a regulatory role in Smc5/6 that is not shared with other SMC complexes. Despite the two RING domains in the Smc5/6 complex are not essential for viability, their mutation leads to enhanced DNA damage sensitivity, indicating they play a crucial role in Smc5/6-dependent DNA damage repair.

Smc5 and Smc6 are the core elements in the Smc5/6 complex, with the Nse2 subunit docking onto the arm domain of Smc5 (Figure 2). The Nse1-Nse3-Nse4 subcomplex, through the N- and C-terminal domains of the Nse4 kleisin subunit, bridges the Smc5 ATPase head and the Smc6 neck [31]. The Nse1 and Nse3 KITE subunits use their N-terminal winged-helix (WHN) domain to heterodimerize and their C-terminal winged-helix (WHC) domain to bind the kleisin subunit [21,22,32]. In humans, the lung immunodeficiency and chromosome breakage syndrome (LICS) has been associated to impaired formation of Nse1-Nse3-Nse4 trimers due to missense mutations in Nse3 WHB region [33]. Nse5 and Nse6, the less conserved subunits of the Smc5/6 complex, form a third subcomplex [34–36]. It is currently unclear how Nse5-Nse6 contribute to Smc5/6 functions. Their localization within the complex is also less well-defined, with reports from

different organisms locating it to the hinge, the arm or the heads [31,37,38]. However, if Smc5/6 bends at the putative elbow region, it is possible that Nse5-Nse6 may be able to interact with the three regions.

Smc5/6 interacts with DNA using different DNA-binding interfaces: Smc5 and Smc6 proteins can bind to DNA through the hinge, the arms and the ATPase head regions [28,39,40]; in addition. Nse1-Nse3 can also interact with DNA [41]. Despite the SMC core and the Nse1-Nse3-Nse4 trimer might be the initial DNA contact point, different NSE subunits have been shown to promote Smc5/6 association with DNA [36,41–44], suggesting that an integer Smc5/6 complex is required for its efficient loading onto chromatin. Smc5/6 can also topologically associate with DNA, using the same ATP-dependent mechanisms as other SMC complexes [45]. This association probably involves switching between the recently observed open and closed arm conformations of the complex and may require the ATP-dependent aperture of the Nse4-Smc6 interface [27,38].

The Smc5/6 complex has been related to wide variety of functions, from the maintenance of genomic integrity, inhibition of viral genome transcription or in plant development (recently reviewed in [11]). Although the Smc5/6 complex is frequently linked to DNA repair function, most Smc5/6 subunits are required for chromosome segregation and are thus essential for viability. In fact, the most striking phenotype after inactivation of *smc5/6* conditional mutants is the failure to separate sister chromatids during the first anaphase. Smc5/6 operates slightly earlier in the cell cycle, in a window that covers from late S phase until the entry into mitosis [46,47]. In budding yeast, the chromosome nondisjunction defects are most obvious in the rDNA array, where Smc5/6 binds throughout the cell cycle [48–50]. The rDNA is a highly repetitive locus that codes for ribosomal RNAs and each repeat contains various obstacles to DNA replication, including a polar replication fork block and high transcription by RNA polymerases I and III. Both features influence the failure to complete rDNA replication and the nondisjunction phenotype of *smc5/6* mutants [50,51]. Although the exact role for Smc5/6 in chromosome replication and segregation is currently unknown, it is probably related to the control of chromosome superhelicity at replication forks [52].

Smc5/6 also maintains a correct balance between accumulation and removal of recombination structures at damaged sites, and *smc5/6* mutants accumulate recombination-dependent structures in the rDNA during a normal cell cycle, and at other locations in response to replication fork damage [48,51,53–55]. In meiosis, Smc5/6 promotes disjunction of homologues by preventing the accumulation of toxic recombination intermediates [56–62]. In mitosis, Smc5/6 directly restrains the activity of Mph1, the budding yeast homologue of the FANCM fork regression enzyme, and activates the Sgs1/BLM helicase to promote dissolution of recombination intermediates [50,63–66]. The Smc5/6 complex is also recruited to DSB [67], where it promotes error-free repair by sister-chromatid recombination [68], and to interstrand crosslinks [36]. In addition, when DSBs occur inside the rDNA repeats or at other heterochromatic regions, Smc5/6 promotes their relocalization into a different compartment of the nucleus to be repaired through homologous recombination, helping to maintain the stability of the affected locus [49,69,70].

Nse2: a SUMO E3 ligase in the Smc5/6 complex

Two features make Nse2 different from other SMC subunits. First, the N-terminal half of Nse2 binds close to the presumed elbow region in the arm domain of Smc5 [13,71] (Figure 2C). This interaction is essential for viability, most likely by performing a structural role on the Smc5/6 complex [71,72]. In addition, the C-

terminal half codes for an SP-RING domain [71,73] that confers SUMO E3 ligase activity [29,74,75]. SUMO is a small protein that can be covalently attached to lysine residues on target proteins through an enzymatic cascade. SUMO is activated by an E1 enzyme and subsequently transferred to an E2 enzyme. Then, the E2 attaches SUMO to the target protein with the assistance of an E3 ligase enzyme [76]. SUMO frequently targets various elements in protein complexes and pathways, showing a synergistic effect [77]. Besides, sumoylation is reversible and highly dynamic due to the action of SUMO proteases [78].

Mutations of conserved residues in the Nse2 SP-RING domain increase the sensitivity to DNA damage in yeast and human cells, revealing a critical role for this SUMO ligase in DNA repair [29,74]. In humans, frameshift mutations in residues located C-terminally of the SP-RING domain have been associated with primordial dwarfism syndrome, severe insulin resistance diabetes and genomic instability [79]. Surprisingly, SP-RING point mutations in mice do not increase the frequency of tumors, suggesting that it may play a minor role during cancer progression [80].

Modification of proteins that localize on chromatin depends on the recruitment of SUMO E3 ligases via DNA-binding domains or interaction with chromatin-associated proteins [81]. Since Nse2 lacks a DNA-interaction interface, its way of access to chromatin is through the Smc5/6 complex [40]. Coherently, the sumoylation and DNA repair activities of Nse2 rely on its stable docking onto a fully active Smc5/6 complex [72]. Notably, loading onto DNA is not sufficient for optimal Nse2-dependent sumoylation, and its E3 activity is further stimulated by direct binding of DNA to a positively-charged patch in the arm domain of Smc5 [40] (Figure 3). Binding of ATP to the head domains most probably remodels the global architecture of the Smc5/6 complex from closed to open conformations. In addition, mutation of a conserved coiled coil disruption next to the Nse2 binding site in Smc5 impairs SUMO ligase activation [72]. This disruption is located in the putative elbow of the Smc5 arm [13], suggesting that proper bending of the arm facilitates activation of Nse2 [72]. The open configuration may expose the DNA sensor in Smc5, enhancing the activity of the SUMO ligase [40]. Changes in Nse2 activity may imply repositioning of the donor SUMO for optimal catalysis through an unknown mechanism, as occurs in other SUMO ligases [82,83]. Besides, the activity of Nse2 is modulated by ATR and PKA-dependent phosphorylation [84,85] and binding to members of the RENi (Rad60-Esc2-NIP45) family of SUMO-like domain proteins, which probably promote recruitment of the Smc5/6 complex to DNA lesions [86–89].

As a SUMO ligase, Nse2 has been linked to various processes, mostly related to the repair of DSBs and damaged replication forks through homologous recombination [90]. To this end, Nse2 targets several factors involved in chromosome organization, replication and DNA damage repair (Figure 3 and Table 1). A first group of Nse2 targets are the three eukaryotic SMC complexes [86,91]. Sumoylation of cohesin promotes sister chromatid cohesion during a normal cell cycle and in response to DNA damage [92–94]. Several subunits in Smc5/6 are also targets of Nse2 themselves. Sumoylation of the coiled coil of Smc5 promotes error-free bypass at damaged replication forks, through a pathway that involves the Mph1 motor protein [66]. In addition, protein group sumoylation of the Smc5/6 complex triggers recruitment of the Sgs1 helicase through two SUMO-interacting motifs (SIM) in Sgs1 [64,65]. Sgs1 is the yeast orthologue of the human Bloom helicase (BLM), which forms part of a three-component complex named STR/BTR (Sgs1-Top3-Rmi1 in yeast, BLM-TOP3A-RMI in humans). STR/BTR is able to catalyze dissolution of recombination intermediates [95,96]. The Smc5/6-STR interaction allows Nse2-dependent sumoylation of various subunits in STR and its concomitant activation [64,65]. In human cells, BLM is also modified and recruited to collapsed replication forks in an Nse2-dependent manner [97,98].

Another group of Nse2 SUMO targets are components of the replication fork [99–103] (Table 1). In budding yeast, the Smc5/6 complex sumoylates various replisome proteins, including Mcm6, a subunit of the replicative helicase, and Pol2, the catalytic subunit of DNA polymerase ε [100]. Pol2 physically interacts with the Smc5/6 complex and is sumoylated by Nse2 during S phase at its N-terminal catalytic domain. In response to replication fork arrest, Pol2 sumoylation increases in a manner dependent on the S phase checkpoint [103]. Modification of Pol2 by the Nse2 ligase contributes to the restart of stalled replication forks preventing recombinational repair and chromosomal rearrangements [101].

The sumoylation activity of Smc5/6 also participates, together with the Slx5/Slx8 SUMO-targeted ubiquitin ligase (STUbL), in the intranuclear dynamics of repetitive loci, by moving heterochromatic DSBs to the periphery of the nucleus. This relocation temporally blocks homologous recombination to avoid erroneous repair events [49,69,104]. In budding yeast, this relocation has been reported in the repetitive rDNA locus, , and seems to require sumoylation of Rad52 [49]. Nse2 also targets two rDNA-specific proteins: the Fob1 protein, which is responsible for the polar arrest of replication forks, and the Rpa135 subunit of the RNA polymerase I complex [66,86], although the role of these modifications is currently unknown. Analogously to chromosome breaks, relocation of damaged replication forks to nuclear pores also relies on Nse2-dependent sumoylation, by targeting the fork-associated proteins RPA, Smc5 and Rad59; STUbL recognizes their sumoylation, moving damaged forks to the nuclear periphery [102,105]. Nse2 also targets the yeast Ndc10 and Bir1 kinetochore proteins, affecting their localization to the mitotic spindles [106].

Finally, Nse2 sumoylates various subunits of the shelterin complex, involved in telomere maintenance [107] (Table 1). This seems to be crucial to prevent senescence in ALT (Alternative-Lengthening of Telomeres) cells, which depend on homologous recombination at chromosome ends for telomere elongation and maintenance.

Nse1: a RING-type subunit with ubiquitin E3 ligase activity?

While the WHN and WHC domain in Nse1 participate in interactions with Nse3 and Nse4, respectively, the most C-terminal part of the protein codes for an NH-RING domain [32] (Figure 2A). The NH-RING resembles the zinc-finger motifs present in ubiquitin E3 ligases [108,109]. They are composed of a central α-helix and two loops containing eight cysteine/histidine residues that coordinate two Zn^{2+} atoms in a cross-brace structure [110]. Nse1 NH-RING motif is evolutionary conserved among species [111], but differs from other classes of RING domains, which typically exhibit a C_3HC_4 pattern of zinc-coordinating residues. Instead, the NH-RING has a C_4HC_3 pattern, similar to the one present in PHD (Plant Homology Domain) fingers [30,112].

Nse1 plays critical roles in the association of Smc5/6 to chromatin [41]. Mutations in the NH-RING partially impair Smc5/6 function, leading to increased DNA damage sensitivity and diminished recruitment of Smc5/6 to damaged sites [30,108,109,113,114]. Interestingly, mutations in zinc-coordinating residues in fission yeast suppress the repair defects of hypomorphic Smc5/6 mutants, probably by preventing the recruitment of dysfunctional complexes to damaged loci [114]. In budding yeast, NH-RING mutants show delayed DNA replication, modest sister chromatid cohesion defects and increased spontaneous chromosome loss events [115].

Interestingly, the NH-RING in Nse1 has been related to two disparately different functions (Figure 2A). On one hand, the yeast NH-RING of Nse1 is necessary for the stability of the Nse1-Nse3-Nse4 subcomplex, and mutations in the NH-RING disrupt the Nse1-Nse4 interaction, resulting in Nse1-Nse3 dimers with defective binding to Nse4 [30,115]. On the other hand, the human NH-RING displays a weak E3 ubiquitin ligase activity *in vitro* that is greatly stimulated in the presence of Nse3 [32]. Nse3 (MAGE-G1 in humans) belongs to the melanoma antigen (MAGE) family of cancer-testis antigens [116–118]. MAGE proteins interact with RING-containing proteins and modulate their ubiquitin ligase activity through the recruitment, or stabilization, of the E2-conjugating enzyme [32,119,120]. Strikingly, Nse1 associates with MAGE-F1 independently from the Smc5/6 complex to regulate the cytosolic iron-sulfur (Fe-S) assembly (CIA) pathway by targeting MMS19 to degradation [121], indicating that its NH-RING domain might cooperate *in vivo* with E2 enzymes to promote ubiquitylation of protein targets. Whether this E3 ligase is active in the context of the Smc5/6 complex is currently unknown, and no Smc5/6 ubiquitylation target has been described to date.

Conclusions

The study of the SP-RING domain in Nse2 has shown that the Smc5/6 complex not only uses its ATPase activity to dynamically associate with DNA and organize chromosomes, but is assisted in this process through sumoylation of specific targets. In addition, recent findings suggest that the activation of this SUMO ligase is intimately connected to the dynamic association of the Smc5/6 complex with DNA. The identification of various Nse2-dependent SUMO targets involved in chromosome organization, DNA replication, recombination and dissolution of joint molecules is in agreement with the sumoylation activity in the Smc5/6 complex directly contributing to sister chromatids disjunction at the end of chromosome replication. On the other hand, there are still many open questions regarding the NH-RING domain in Nse1. It is currently unknown if it displays any ubiquitin ligase activity at all within the context of an Smc5/6 complex, the identity of its putative targets, the E2 enzymes it might bind to, the types of ubiquitin chains it might promote or even the outcome of such modifications. Indeed, it is possible that although the human NH-RING domain can sustain protein ubiquitylation *in vitro*, its functions within the Smc5/6 complex might be limited to protein-protein interactions and proper Smc5/6 assembly. Future analyses, including the structural analysis of the NH-RING domain in the Nse1-Nse3-Nse4 subcomplex and proteomic studies of the Nse1-dependent ubiquitinome, will be required to further decipher the role for this RING domain in the Smc5/6 complex.

Perspectives

(i) Chromosome organization by SMC complexes, and post-translational modification of chromosome-bound proteins are two fundamental aspects of chromosome replication, segregation and repair that extraordinarily converge in the Smc5/6 complex.

(ii) The SP-RING domain in Nse2 assists the chromosome dynamics and organization functions of the Smc5/6 complex through sumoylation of various targets, mostly identified in yeast. In contrast, the NH-RING domain in Nse1 has structural roles in Smc5/6 assembly and an E3 ubiquitylation activity in the complex has not been reported.

(iii) Several aspects of Smc5/6 architecture and function are missing and will need to be addressed in the future, including a detailed understanding of its molecular roles in chromosome topology, loop extrusion and chromatin entrapment, and the specific roles played by the different subunits, including the NH-RING domain in Nse1 and the less well known Nse5-Nse6 subunits. In addition, it will be very interesting to unravel how the two RING type subunits contribute to the newly emerging roles of the Smc5/6 complex.

Figure Legends

Figure 1. Structure of SMC complexes and potential mechanism for DNA entrapment and loop extrusion.

A. General structure of SMC proteins, including the Hinge domain (upper part), the elongated coiled coil or Arm domain, and the ATPase head domain (bottom part). Note the position of Elbow and Neck regions in the arm domain, which are responsible for bending of the molecule and kleisin interaction, respectively. **B**. Structure of SMC complexes. Two SMC monomers dimerize through their hinge domain. The kleisin subunit bridges the monomers at the other end of the molecule, contacting the head domain of one monomer through it C-terminus and the neck domain in the other monomer though it N-terminus. Additionally, other non-kleisin subunits, known as HAWKs (in cohesin and condensin) or KITEs (in Smc5/6 and prokaryotic SMC complexes) associate with the kleisin and the heads. The arm domain can bend at the elbow region in the arm domain, thus folding the SMC molecule and changing its conformation. **C**. ATPase binding promotes the engagement of the head domains, opening of the arm domain and opening a gate, most probably by removing the kleisin-neck interaction. **D**. Binding of DNA to different regions in the SMC molecule and the ATPase cycle induces opening of the arm domain and capture of a DNA loop inside the complex. Subsequent hydrolysis of ATP changes the conformation of the ATPase heads, the arm domain and closes the gate. These conformational changes may promote the topological entrapment of DNA inside the SMC molecule and the extrusion of a loop.

Figure 2. Architecture of the Smc5/6 complex and structure of its RING-domain subunits.

A. Structure of the human Nse1-Nse3 dimer (PDB 5WY5). Nse1 (green) and Nse3 (light yellow) interact with each other through their WHN domain and with the Nse4 protein through the WHC domain. Note the presence of the NH-RING domain protruding out of the Nse1-Nse3 dimer (encircled with a dotted line), with roles in Smc5/6 assembly and ubiquitylation. **B**. Architecture of the Smc5/6 complex. Nse1 and Nse3 bind to Nse4 through their WHC domain. Nse4 binds to the Smc5 head using its C-terminal domain, and to the neck in Smc6 through its N-terminal domain, bridging the lower part of the molecule. Nse2 binds to the middle part of the Smc5 arm domain, close to the presumed elbow region. The Nse5-Nse6 subcomplex probably binds to the lower part of the arm domain. **C**. Structure of the yeast Nse2 subunit in complex with the arm domain of Smc5 (3HTK). Nse2 (green) interacts with Smc5 (red) using a helix bundle in its essential N-terminal domain. The SP-RING domain (encircled with a dotted line), responsible for the SUMO ligase activity of Nse2, is located in upper lobe, which also includes a C-terminal alpha helix.

Figure 3. Model for DNA-dependent activation and targets of the SUMO ligase in the Smc5/6 complex.

Left, **DNA binding of Smc5/6**. Different regions in the Smc5/6 complex, the Nse1-Nse3 subunits, the Nse5-Nse6 subunits (not shown in the figure), the heads, the hinge and the arm domains, mediate binding and recruitment of the complex to damaged sites, including DSBs, interstrand crosslinks and replication forks. **Middle**. **Speculative model for topological loading of Smc5/6**. Binding to ATP might promote engagement of head domains, the transition from closed to open conformations in Smc5/6 and topological entrapment of the complex on DNA, probably through opening of an Nse4-Smc6 gate. Loaded Smc5/6 molecules might promote loop extrusion, but it is currently unknown how Smc5/6 loading and its ATPase activity alter DNA topology. **Right**. **SUMO ligase activation in the Smc5/6 complex**. Once loaded, DNA interacts with a positively charged patch in the Nse2-binding site of the Smc5 arm and DNA, enhancing the SUMO ligase activity of Nse2 towards its targets. The presence of conserved proline residues in the putative elbow of the Smc5 arm is required for full Nse2 activity, suggesting that bending of the arm participates in SUMO ligase activation [72]. Once loaded, Nse2-dependent sumoylation cooperates with the Smc5/6 complex in various tasks, including chromosome organization and disjunction, fork restart and relocation of damaged sites to a different nuclear compartment.

Table 1. List of known Nse2 SUMO targets.

Protein	Function	Organisms	References
Smc1	Cohesin complex	*S. cerevisiae*	[72,84,91]
Smc3	Cohesin complex	*S. cerevisiae*	[86,91]
Scc1	Cohesin complex	*S. cerevisiae*, humans	[86,92–94,122]
SA1	Cohesin complex	Humans	[122]
Smc2	Condensin complex	*S. cerevisiae*	[86,91]
Smc4	Condensin complex	*S. cerevisiae*	[86]
Smc5	Smc5/6 complex	*S. cerevisiae*	[66,72,75,86,100]
Smc6	Smc5/6 complex	*S. cerevisiae, S. pombe*, humans	[29,74,84]
Nse2	Smc5/6 complex	Humans	[29]
Nse3	Smc5/6 complex	*S. cerevisiae, S. pombe*	[74]
Nse4	Smc5/6 complex	*S. cerevisiae, S. pombe*	[72,123]
Sgs1/BLM	STR/BTR complex	*S. cerevisiae*	[64,65,98]
Top3	STR/BTR complex	*S. cerevisiae*	[64]
Rmi1	STR/BTR complex	*S. cerevisiae*	[65]
Pol2	DNA replication	*S. cerevisiae*	[100,101,103]
Mcm6	DNA replication	*S. cerevisiae*	[100]
Rfa1	DNA replication	*S. cerevisiae*	[102]
Rfa2	DNA replication	*S. cerevisiae*	[102]
Rfa3	DNA replication	*S. cerevisiae*	[102]
Rad59	DNA repair	*S. cerevisiae*	[102]
TRAX	DNA repair	Humans	[29]
Ku70	End joining	*S. cerevisiae*	[75]
Tin2	Shelterin complex	Humans	[107]
Trf1	Shelterin complex	Humans	[107]
Trf2	Shelterin complex	Humans	[107]
Rap1	Shelterin complex	Humans	[107]
Rpa135	RNA Pol I	*S. cerevisiae*	[86]
Fob1	rDNA	*S. cerevisiae*	[66]
Ndc10	Kinetochore	*S. cerevisiae*	[106]
Bir1	Kinetochore	*S. cerevisiae*	[106]
Smyd1	Myogenesis	Mice	[124]

List of Nse2 SUMO-targets validated in SUMO purification assays using *nse2* mutants, or in western blot analysis in cells over-expressing SUMO and Nse2. The list does not include other potential targets identified in proteomic approaches or through indirect evidence.

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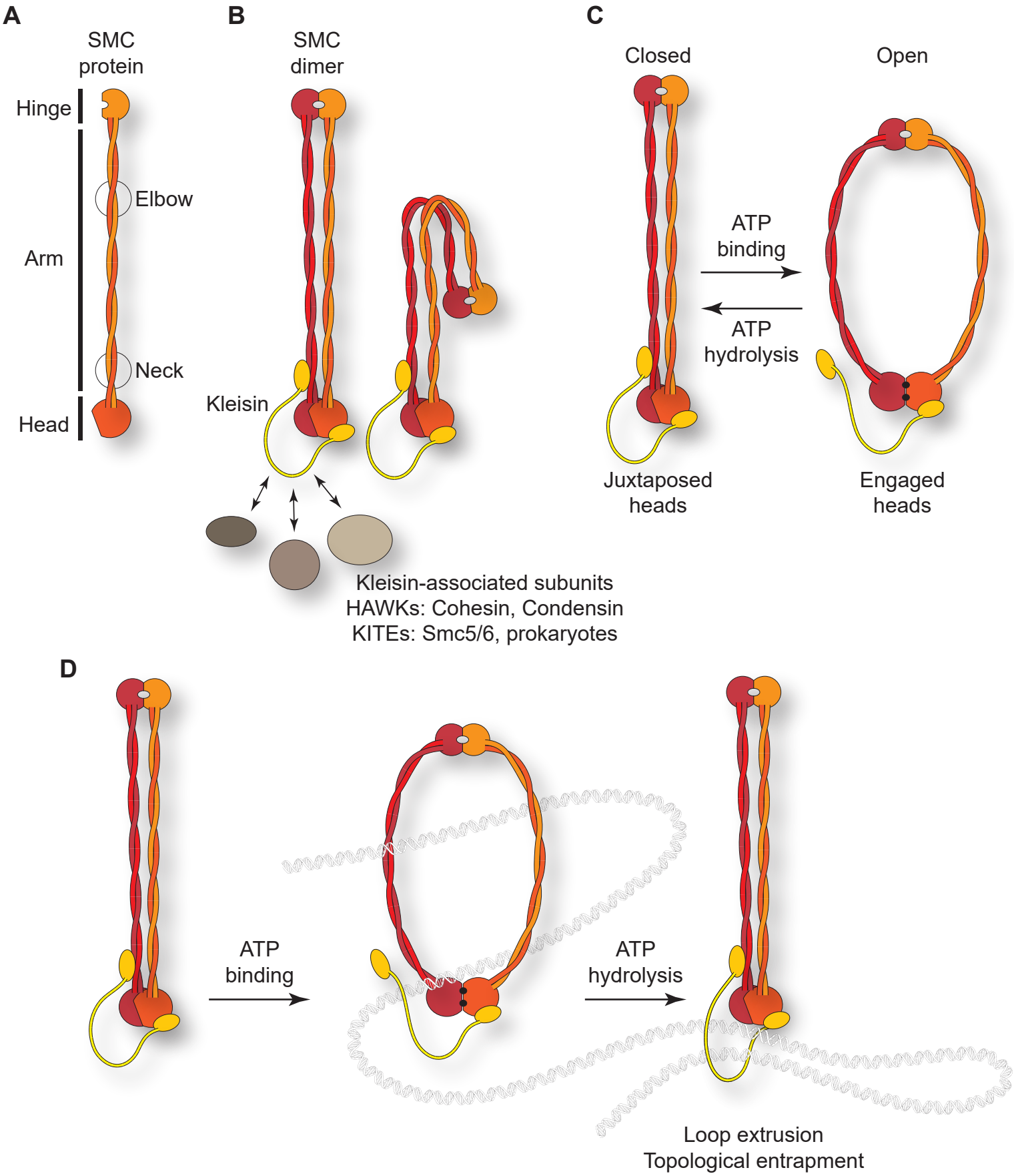
Author contributions

J. T.-R. and R. S.-S. wrote the manuscript.

Declaration of Interests
The authors declare no competing interests.

Acknowledgements
We thank all members of the Cell Cycle lab for useful discussions.

Funding
Work in the J.T.-R. lab is supported by grant and PGC2018-097796-B-I00 from Ministerio de Ciencia, Innovación y Universidades and grant 2017-SGR-569 from AGAUR-Generalitat de Catalunya. R.S.-S. is a recipient of an FPU16/07021 fellowship from Ministerio de Ciencia, Innovación y Universidades. The IRBLLEIDA Institute is part of CERCA Programme/Generalitat de Catalunya.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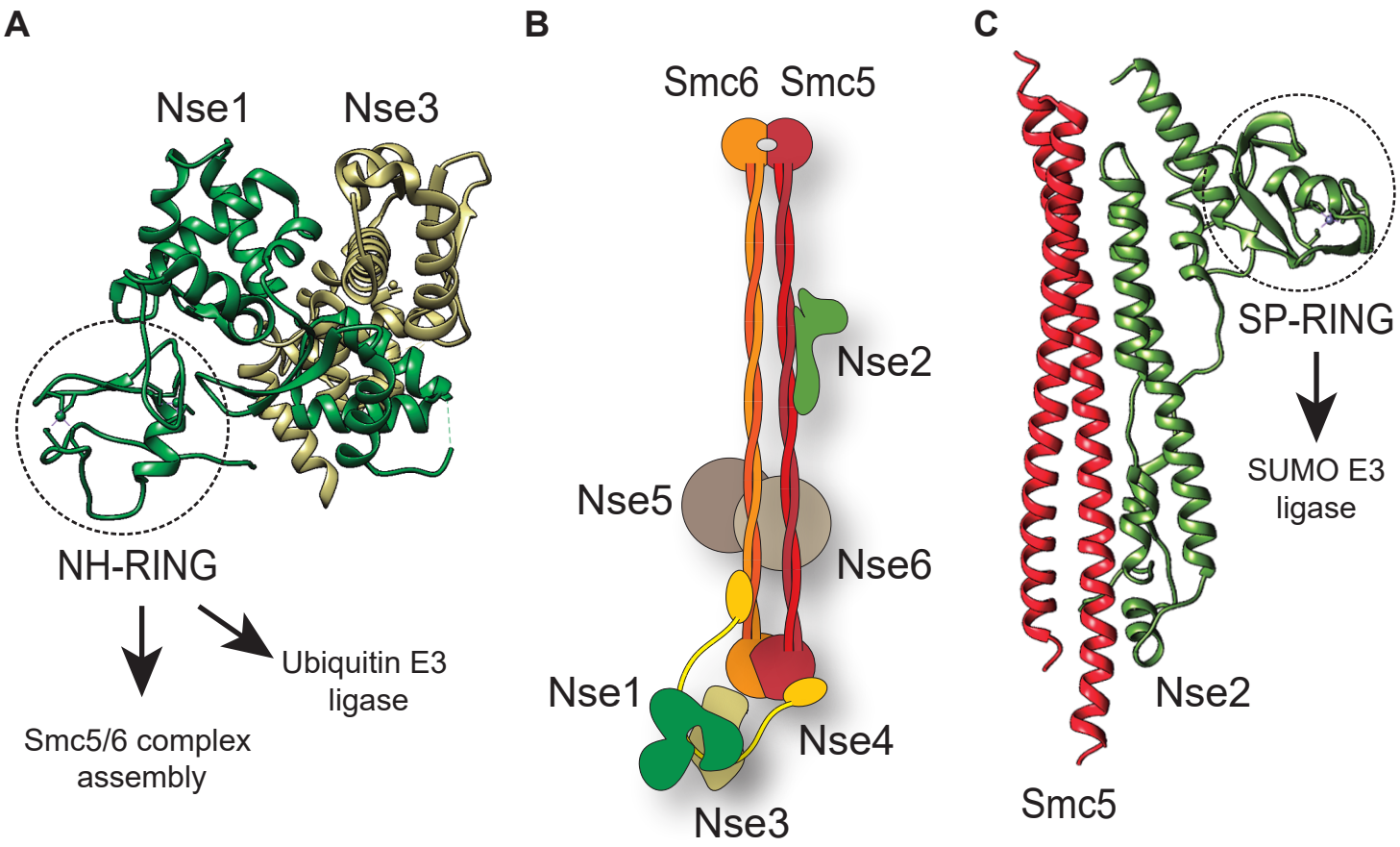
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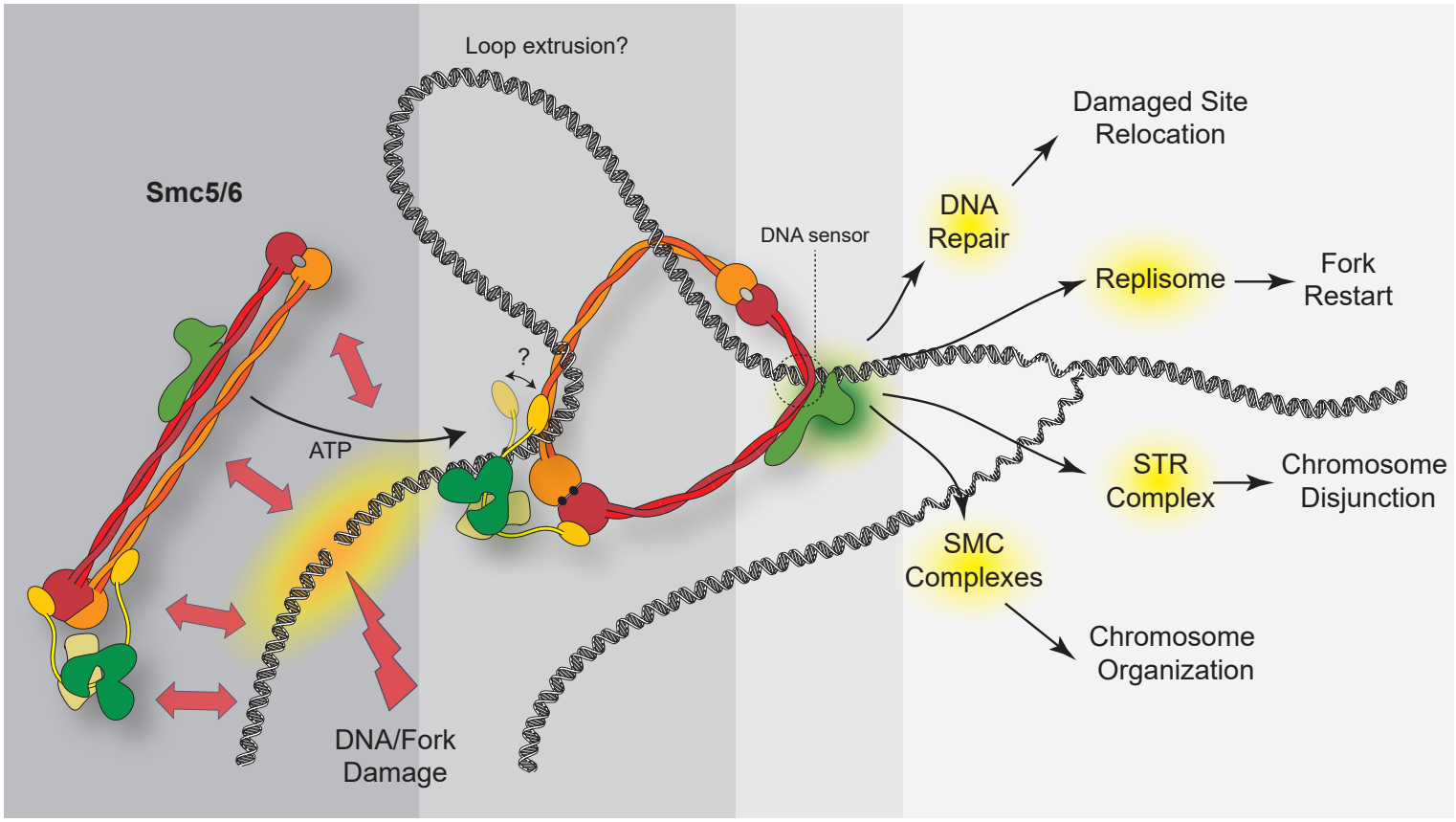
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Organization
DNA/Fork
Damage
DNA binding
Topological loading
SUMO ligase
activation